



Cuticular lipids of female solitary bees, *Osmia lignaria* Say and *Megachile rotundata* (F.) (Hymenoptera: Megachilidae)

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ABSTRACT

The cuticular lipids of the cavity-nesting adult female solitary bees, *Osmia lignaria* Say and *Megachile rotundata* (F.) (Hymenoptera: Megachilidae), were analyzed by gas chromatography (GC) and combined GC–mass spectrometry. The cuticular lipids of these female bees are mainly consisted of hydrocarbons. For *O. lignaria*, nearly 64% of the cuticular lipids were C₂₅–C₃₁ mono-alkenes. For *M. rotundata*, 48% of the cuticular lipids were C₂₃–C₃₃ alkanes with nearly the same quantities of the same chain-length mono-alkenes (45%). For the mono-alkenes of *O. lignaria*, 14 mono-alkene constituents were identified, with two of these, 9-heptacosene and 7-nonacosene, comprising 67% of the total alkene distribution. For *M. rotundata* females, the mixtures of mono-alkenes were more complex with 26 constituents identified and quantified. For the *M. rotundata* mono-alkenes, 57% of the total composition consisted of the three alkenes, 7-pentacosene, 9-pentacosene and 7-heptacosene. For both bee species, small quantities of C₄₀–C₄₈ wax esters were also characterized with the major components possessing a C₁₈ mono-unsaturated fatty acid (9-octadecenoate) moiety esterified to even-carbon number (C₂₂–C₃₀) fatty alcohols. The possible role of these cuticular lipids as nest recognition chemicals is discussed in light of nesting behavior of managed crop pollinators.

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1. Introduction

The primary function of insect cuticular lipids is to provide a moisture barrier and protection from harmful environmental elements (Hadley, 1984). However, these same lipids are known to be semiochemicals that serve as inter- and intra-specific recognition cues (Howard, 1993; Singer, 1998). The cuticular lipids of a few solitary bees have been examined for their role as sex pheromones or for indicating mating status (e.g., Paulmier et al., 1999; Ayasse et al., 2001; Simmons et al., 2003; Mant et al., 2005). A few studies have also shown that lipids from glandular secretions are also found in linings of solitary bee nest cavities (Hefetz et al., 1979; Cane, 1983; Espelie et al., 1994; Guédot et al., 2005, 2006). For the blue orchard bee *Osmia lignaria* Say and the alfalfa leafcutting bee *Megachile rotundata* (F.) (Hymenoptera: Megachilidae), the chemical signals of these nest linings allow the nesting female bee to discriminate between her nest and that of others in a nesting aggregation (Guédot et al., 2005, 2006). Behavior evidence suggests that the female applies the lipids from her abdominal region, but the exact origin of these lipids is unknown.

Spring-flying *O. lignaria* and summer-flying *M. rotundata* are economically important as commercial pollinators for orchard fruit and alfalfa seed production, respectively. Megachilid bees are solitary and not social, like honey bees. These particular solitary bees are gregarious; they may nest near one another in large numbers, but each female is fertile and builds her own nest. The females of these species are highly efficient pollinators, being driven by the instinctive behavior to collect large amounts of pollen and nectar for the provisioning of their brood and, therefore, pollinate high numbers of flowers (Bosch and Kemp, 2001). The female bees rely on visual and/or olfactory cues to locate their nests (Guédot et al., 2007). For brood production, females create nests in pre-existing holes, arranging a linear row of cells in which they deposit a mixture of nectar and pollen in the form of provision mass (Klostermeyer and Gerber, 1969; Richards, 1984; Torchio, 1989; Pitts-Singer, 2007). Within nesting cavities, *M. rotundata* females line each nesting cell with cut leaf pieces and partition the cells with leaf discs (Richards, 1984), whereas *O. lignaria* females separate each cell from the next with a mud partition (Torchio, 1991; Bosch and Kemp, 2001). Emphasis has been placed on acquiring a better understanding of the nesting behavior of these pollinator bees to provide adequate nesting resources and quality populations to commercial growers.

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Laboratory and greenhouse experiments on the nesting behaviors of *M. rotundata* and *O. lignaria* using clear glass artificial nesting tubes have been conducted and the subsequent chemical analysis of tube contents compared to the chemical analysis of cuticular lipids from the test female bees (Guédot et al., 2005, 2006). Observations have indicated that nesting females drag their abdomen along the tube before exiting, spiraling inside the tube, and sometimes depositing tiny fluid droplets from the tip of the abdomen (Guédot et al., 2006). Abdominal fluids containing nest recognition chemicals could originate from the Dufour's gland (Hefetz et al., 1990; Hefetz, 1992). The source for *O. lignaria* and *M. rotundata* nest recognition chemicals could also be from cuticular lipids transferred from actively-nesting females by the deliberate or incidental cuticular contact with walls of the nesting cavity. In chemical analyses of nesting tube contents for female *O. lignaria* (Guédot et al., 2006) and female *M. rotundata* (Guédot et al., 2005), we identified mixtures of lipids that probably originated from the nesting female and not from other tube components, such as pollen for provision or other nesting materials (i.e., *M. rotundata* leaf cuttings or *O. lignaria* mud) (Guédot et al., 2005, 2006).

Thus, for the purpose of determining the involvement of cuticular lipids as nest recognition chemicals, we identified the cuticular lipids extracted from the cuticular surfaces and compare lipid distributions for newly emerged adult female *O. lignaria* and *M. rotundata*. Cuticular lipids extracted from newly emerged, lab-maintained females should be free of chemical contaminants brought to the nest by foraging bees, such as plant and nest-building materials. The type and amounts of cuticular lipids found on these bees may also suggest how these bees thrive under the environmental conditions characteristic of the season when they are active.

2. Materials and methods

2.1. Insects and extraction procedures

Overwintering, cocooned *M. rotundata* prepupae (within their nesting cells) and cocooned, dormant *O. lignaria* adults were shipped (under chilled conditions) from the USDA-ARS Pollinating Insect Research Laboratory, Logan, UT to the USDA-ARS Biosciences Research Laboratory, Fargo, ND and held at 4 °C until use. Cocoons were warmed to room temperature and held for 1–7 days so as to facilitate emergence of *O. lignaria* adults. For emergence of *M. rotundata* adults, prepupae in their nesting cells were incubated at 29 °C for 20–30 days. After emergence, adults of both species were held 12–24 h in glass vials before solvent extraction of their cuticular lipids. The cuticular lipids were removed from ice-chilled individual *O. lignaria* adults by gentle extracting (at room temperature) in 8 mL hexane for 60 sec, then a quick rinse in 4 mL hexane, followed by a 30 s extraction in 8 mL chloroform (CHCl_3) and a quick rinse in 4 mL CHCl_3 . The same timed protocol was used for cuticular lipid extraction of *M. rotundata* adult bees, but with smaller volumes of 5 mL and 3 mL for initial extractions and quick rinses, respectively. For the brief CHCl_3 extractions and rinses, chloroform with amylene as a stabilizer (Chloroform (without ethanol), Honeywell Burdick & Jackson, Muskegon, MI) was used. The use of CHCl_3 that has ethanol (0.5–1.0%) as stabilizer increases the polarity of the extracting solvent and increases the chances for damage to the exocuticle, and hence, release of contaminating internal lipids. Also, the short exposure to the organic solvents was preferred because of concerns of extracting Dufour's gland or any other exocrine gland material.

2.2. Chromatography and structural identifications

Gas chromatography–flame ionization detector (GC–FID) analyses of *O. lignaria* samples were performed using an Agilent 6890

gas chromatograph equipped with a temperature- and pressure-programmable on-column injector (Agilent Technologies, Palo Alto, California), and an Alltech AT™-1HT capillary column (0.25 mm \times 15 m \times 0.1 μm) (Alltech Associates, State College, PA, USA). *M. rotundata* samples were analyzed by GC–FID using a J&W Scientific DB-1MS capillary column (0.2 mm \times 12.5 m \times 0.33 μm). Chromatography conditions for both column types were the same: the column oven temperature was held at 75 °C for 0.5 min, increased to 225 °C at 25 °C/min, next increased at 10 °C/min to 300 °C, then increased at 25 °C/min to 320 °C and held for 45 min. Samples were introduced onto the column via a 2.0 m retention gap of uncoated, deactivated fused silica with the hydrogen carrier at 20 psig. After 0.5 min, the pressure was reduced to 7 psig, and then increased at 1 psi/min to 30 psig where it was held until the end of the run.

For both species, GC–mass spectrometry (MS) was performed on a Hewlett Packard Model 5890A gas chromatograph equipped with a temperature- and pressure-programmable on-column injector (Agilent Technologies, Palo Alto, CA, USA) and a 1 m retention gap, connected to a J&W Scientific DB-1MS capillary column (0.2 mm \times 12.5 m, 0.33 μm phase thickness) (Folsom, California) coupled to an HP 5970B quadrupole mass selective detector (Agilent Technologies, Palo Alto, CA, USA). The helium carrier gas flow was a constant 0.75 mL/min. The column temperature was initially held at 150 °C for 4 min, programmed to 320 °C at 4 °C/min where it was held until all observable peaks eluted.

2.3. Double bond location for alkenes

For determination of double bond location, the mono-alkenes from the cuticular lipids of *O. lignaria* and *M. rotundata* were analyzed by GC–MS as their dimethyl disulfide (DMDS) adducts. The hydrocarbon fractions were purified from the cuticular lipid extracts of individual females by passing extracts through a 0.5 \times 10 cm silica gel column with hexane. A portion of each hydrocarbon fraction (30–50%) was transferred to 500 μL glass Reacti-vials® and the solvent evaporated under nitrogen. To each vial was added 100 μL hexane, 100 μL DMDS, and 20 μL of an iodine solution (60 mg of iodine in 1 mL of dry diethyl ether). The reaction mixture was heated overnight at 60 °C, cooled and then mixed with 200 μL of a 5% solution of sodium thiosulfate in water. The aqueous layer was then extracted several times with hexane. The combined hexane layers were evaporated and stored under argon at –20 °C. Mass spectra for the dimethyl disulfide adducts were obtained by GC–MS using the J&W Scientific DB-1MS capillary column and the temperature- and pressure-program method as described above.

2.4. Quantification of lipids and derivatives

Quantities of the saturated hydrocarbons and all wax esters were determined using the integrated peak area data from the FID response to increasing quantities (0.39–200 ng) of the authentic standard, *n*-octacosane as previously described (Buckner et al., 1999). The FID response of the authentic standard, 1-docosene consistently produced an FID signal that was 90% of the signal produced by the same mass of *n*-octacosane. So the peak areas of the mono-alkenes were divided by 0.9 before calculating their mass using the *n*-octacosane standard curve. The calculations for the percentage composition of mono-alkene positional isomers for each chain length were based on the relative abundance of the major mass spectral ion for the dimethyl disulfide derivative. These percentages were applied to GC–FID mass data for each mixed-isomer alkene peak to determine the quantity of each positional isomer. Those values were used to calculate the percentage composition of the alkene isomers for each of the twelve *O. lignaria* and eight *M. rotundata* females.

3. Results

3.1. Chromatography

Subsequent GC–FID and GC–MS analyses of the cuticular lipid extracts of both species contained the same major lipid classes: hydrocarbons, with lesser amounts of wax esters and detectable amounts of methyl-branched alkanes. Chromatographic profiles from GC–FID analyses revealed major components as long-chain alkanes and mono-alkenes, with smaller quantities of unsaturated wax esters

Table 1

Percent composition (mean \pm SD) for cuticular lipids of *O. lignaria* and *M. rotundata* female adults.

Peak number ^b	Percent composition ^a		Component(s) ^c
	<i>O. lignaria</i>	<i>M. rotundata</i>	
1		0.3 \pm 0.1	7-tricosene ^d
2	1.7 \pm 0.5	2.3 \pm 0.5	Tricosane
3	0.2 \pm 0.1		3- and 5-methyl tricosane
4			Contaminant
5	0.3 \pm 0.1	0.2 \pm 0.1	Tetracosane
6	4.3 \pm 1.5	8.5 \pm 1.4	12-, 11-, 10- and 9-pentacosene
7	4.8 \pm 1.0	13.2 \pm 2.8	7-pentacosene
8	0.1 \pm 0.1	1.8 \pm 0.8	5-pentacosene
9	16.5 \pm 1.2	17.9 \pm 2.5	Pentacosane
10	0.2 \pm 0.0	0.1 \pm 0.1	9-, 11-, 13- and 5-methyl pentacosane
11	1.4 \pm 0.2	0.6 \pm 0.3	Hexacosenes
12	0.4 \pm 0.0	0.5 \pm 0.2	Hexacosane
13	29.4 \pm 2.9	6.6 \pm 1.1	13-, 11-, 10- and 9-heptacosene
14	6.4 \pm 1.2	5.7 \pm 0.6	7-heptacosene
15	0.2 \pm 0.1	2.1 \pm 0.7	5-heptacosene
16	6.8 \pm 0.8	11.9 \pm 2.2	Heptacosane
17		0.3 \pm 0.5	Octanoyl 9-octadecenoate (26:1 WE) ^f
18	0.2 \pm 0.0	0.2 \pm 0.2	13-, 11- and 9-methyl heptacosane
19		0.2 \pm 0.3	Octanoyl octadecanoate (26:0 WE) ^f
20	0.5 \pm 0.1	t	Octacosenes
21	0.2 \pm 0.1	0.3 \pm 0.1	Octacosane
22	2.6 \pm 0.7	2.0 \pm 0.6	13-, 11-, 10- and 9-nonacosene
23	11.1 \pm 2.3	1.4 \pm 0.3	7-nonacosene
24		0.5 \pm 0.2	5-nonacosene
25	2.8 \pm 0.5	8.3 \pm 2.2	Nonacosane
26	0.1 \pm 0.0	0.2 \pm 0.1	13-, 11- and 15-methyl nonacosane
27	0.2 \pm 0.1		7-triacontene ^d
28	0.2 \pm 0.1	0.2 \pm 0.1	Triacotane
29	0.1 \pm 0.1	0.2 \pm 0.1	13-, 11-, 10- and 9-hentriacontene
30	1.9 \pm 0.8	1.2 \pm 0.3	7-hentriacontene ^e
31	1.9 \pm 0.4	4.7 \pm 1.1	Hentriacontane
32	0.1 \pm 0.0		13-, 15- and 11-methyl hentriacontane
33			Contaminant
34	0.1 \pm 0.0	0.1 \pm 0.0	Dotriacontane
35		1.0 \pm 0.5	7-tritriacontene ^d
36	0.3 \pm 0.1	1.3 \pm 0.3	Tritriacontane
37	0.1 \pm 0.1		15- and 17-methyl tritriacontane
Wax esters^f			
40:1 WE	0.5 \pm 0.1	0.1 \pm 0.1	Docosanoyl 9-octadecenoate
42:1 WE	1.7 \pm 0.4	1.0 \pm 0.3	Tetracosanoyl 9-octadecenoate
44:1 WE	1.0 \pm 0.3	0.9 \pm 0.3	Hexacosanoyl 9-octadecenoate
46:1 WE	1.2 \pm 0.5	2.3 \pm 0.8	Octacosanoyl 9-octadecenoate
48:1 WE	t	2.0 \pm 1.4	Triacotanoyl 9-octadecenoate

t = trace amount (mean < 0.1).

^a Percent compositions were calculated as mean \pm SD values ($n = 8$, *M. rotundata*; $n = 12$, *O. lignaria*).

^b Peak number assignment as indicated in chromatograms in Fig. 1A and B.

^c For the percent distribution of co-eluting double bond positional isomers for peaks 6, 13, 22 and 29, see Table 2. These positional isomers are listed in order of elution within the peak (as determined by GC–SIM analysis). For the methyl alkanes (peaks 3, 10, 18, 26, 32 and 37), positional isomers are listed in order of abundance.

^d The major mono-alkene is the 7-isomer with detectable amounts of the 5-, and 9-, 10-isomers for peak 1, and 9-, 10-isomers for peaks 27 and 35.

^e The major mono-alkene is the 7-isomer for *O. lignaria* and *M. rotundata* (peak 30) with detectable amounts of the 9-, 11- and 13-, 15-isomers, respectively.

^f The numbers to the left and the right of the colon for wax esters are the number of carbons and the number of double bonds in the acyl moiety, respectively. For each wax ester carbon chain length, the major isomer is listed.

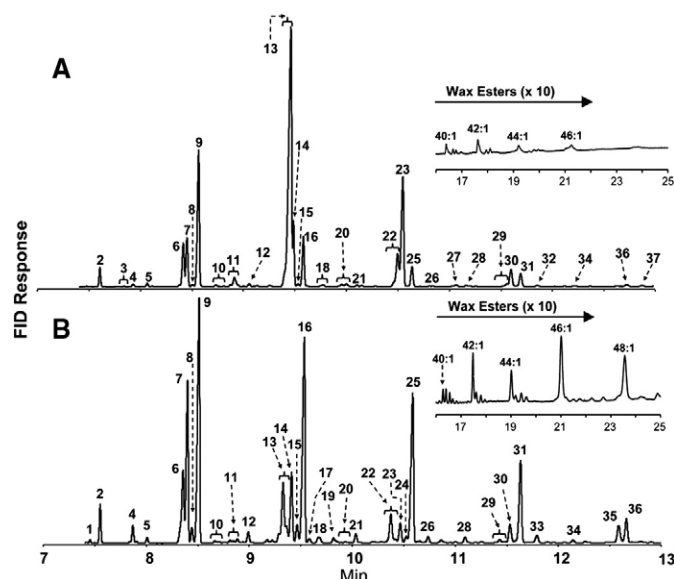


Fig. 1. GC–FID chromatograms of hydrocarbon (7–13 min) and wax ester (16–25 min) regions for cuticular lipids from A, a typical 1–2 day-old female *O. lignaria* and B, a typical 1–2 day-old female *M. rotundata*. The FID responses for the wax ester regions are increased by a factor of 10. See Table 1 for identification of lipid component(s) for each numbered response peak. For wax ester components, the numbers to the left and the right of the colon are the number of total carbons for both the acyl and alcohol moieties and the number of double bonds in the acyl moiety, respectively. Brackets are used to encompass the slight differences in retention of isomers in mixed-isomer peaks.

(Table 1, Fig. 1A and B). Quantities (ng) of each component were determined from the GC–FID data by comparing the peak response for known quantities of lipid standards. The total amount of lipid from each female bee was calculated by summing the ng quantities for all integrated GC–FID peaks. The lipid totals for each female bee varied considerably. For 12 female *O. lignaria*, the total lipid averaged 121.4 ± 50.8 $\mu\text{g}/\text{female}$ (mean \pm SD) and ranged between 61.8 and 230.2 $\mu\text{g}/\text{female}$. For eight *M. rotundata* females, the amount of lipid averaged 56.7 ± 18.2 $\mu\text{g}/\text{female}$ and ranged between 31.4 and 91.3 $\mu\text{g}/\text{female}$.

GC–FID and GC–MS analyses of the *O. lignaria* cuticular lipids revealed that 95% of lipids were comprised of hydrocarbons (63.7% unsaturated and 31.5% saturated components) and 4.6% as wax esters (Fig. 1A). The cuticular lipids of *M. rotundata* were also mainly hydrocarbons (94%) with small quantities of wax esters (6.7%) (Fig. 1B). The hydrocarbons were near equal mixtures of *n*-alkanes (47.8%) and mono-alkenes (45.1%). For both species, the mixtures of mono-alkenes, *n*-alkanes and very small quantities of mono-methyl-branched alkanes were mainly odd-numbered carbon chains ranging from C_{23} – C_{33} and the percentage compositions for cuticular lipid components are presented in Table 1. For *O. lignaria* cuticular lipids, the major component (peak 13) was a mixture of positional isomers of 13-, 11-, 10-, and 9-heptacosene, (C_{27} alkenes) and constituted 29.4% of the cuticular lipids. Other major components, in decreasing order of abundance were C_{25} alkane (16.5%), C_{29} alkene (7-nonacosene, 11.1%), C_{27} alkane (6.9%), C_{27} alkene (7-heptacosene, 6.4%), C_{25} alkene (7-pentacosene, 4.8%) and C_{25} alkenes (12-, 11-, 10-, 9-pentacosene, 4.3%) (Table 1, Fig. 1A). In contrast, the cuticular hydrocarbon components of *M. rotundata* females consisted of substantial quantities of alkanes: C_{25} (17.9%), C_{27} (12.1%), C_{29} (8.3%), C_{31} (4.7%) and lesser amounts of C_{23} and C_{33} alkanes (Table 1, Fig. 1B). 7-Pentacosene (C_{25} , 13.2%) was the major mono-alkene and lesser amounts for the C_{25} positional isomers peak (12-, 11-, 10-, 9-pentacosene, 8.5%) and the peaks for the C_{27} alkene isomers (13-, 11-, 10-, 9-heptacosenes, 6.8%; 7-heptacosene, 5.7%).

The minor quantities of wax esters as cuticular lipid components for both *O. lignaria* and *M. rotundata* female adults were identified as homologous series of C_{40} – C_{48} compounds. The major wax ester peak

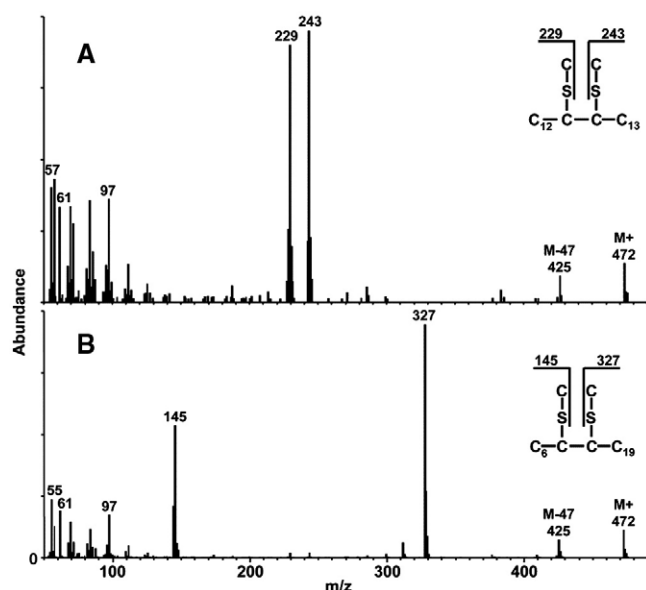


Fig. 2. Mass spectra of the bis(methylthio)alkane derivatives of A, 13-heptacosene and B, 7-heptacosene: peaks 13 and 14, respectively, for mono-alkenes from *M. rotundata* adult females (see Fig. 1B).

for each chain-length series was identified as an alkanyl alkenoate with a $C_{18:1}$ mono-unsaturated fatty acid (octadecenoate) moiety esterified to even-carbon number (C_{22-30}) fatty alcohols (Table 1, Fig. 1A and B). *M. rotundata* cuticular lipids also consisted of small quantities of C_{26} alkanyl alkenoate and C_{26} alkanyl alkenoate (Table 1, Fig. 1B, peaks 17 and 19, respectively).

Table 2
Percent composition (mean \pm SD) for alkene isomers of *O. lignaria* and *M. rotundata* female adults.

Peak number ^b	Percent composition ^a		Component
	<i>O. lignaria</i>	<i>M. rotundata</i>	
1		0.5 \pm 0.2	7-tricosene
6		1.1 \pm 0.1	12-pentacosene
6		1.0 \pm 0.1	11-pentacosene
6		1.7 \pm 0.2	10-pentacosene
6	7.1 \pm 2.0	13.4 \pm 1.4	9-pentacosene
7	7.8 \pm 2.2	30.4 \pm 3.2	7-pentacosene
8	0.1 \pm 0.1	4.1 \pm 0.4	5-pentacosene
11	1.4 \pm 0.3	0.3 \pm 0.1	9-hexacosene
11	0.3 \pm 0.1	0.5 \pm 0.2	7-hexacosene
13		6.4 \pm 0.6	13-heptacosene
13		1.4 \pm 0.1	11-heptacosene
13		1.7 \pm 0.2	10-heptacosene
13	48.5 \pm 2.2	2.5 \pm 0.2	9-heptacosene
14	8.0 \pm 0.4	13.2 \pm 1.2	7-heptacosene
15	0.5 \pm 0.1	6.5 \pm 0.6	5-heptacosene
20	0.3 \pm 0.1		7-octacosene
22		3.0 \pm 0.7	13-nonacosene
22		0.3 \pm 0.1	11-nonacosene
22		0.3 \pm 0.1	10-nonacosene
22	3.6 \pm 0.7	0.3 \pm 0.1	9-nonacosene
23	18.4 \pm 3.6	3.6 \pm 0.8	7-nonacosene
24		1.5 \pm 0.3	5-nonacosene
27	0.2 \pm 0.1		7-triacontene
29		0.4 \pm 0.1	13-hentriacontene
29		t	10-hentriacontene
29	0.2 \pm 0.1	t	9-hentriacontene
30	2.9 \pm 1.3	2.2 \pm 0.6	7-hentriacontene
35		2.1 \pm 1.2	7-tritriacontene

t = trace amount (mean < 0.1).

^a Percent composition for alkenes were calculated as mean \pm SD values ($n = 8$, *M. rotundata*; $n = 12$, *O. lignaria*).

^b Peak Number assignment as indicated in chromatograms in Fig. 1A and B. Multiple alkene isomers within a given Peak Number are listed in order of their elution from the GC column.

3.2. Double bond position and percentage distribution for alkenes

Double bond positions for the mono-alkene components were determined by GC-MS analyses of their dimethyl disulfide derivatives. Mass spectra were characterized by major fragment ions formed by cleavage of the doubly activated bond formed by derivatization producing α,β -bis(methylthio)alkanes (Francis and Veland, 1981). For example, the mass spectrum for the bis(methylthio)alkane derivatives of 13-heptacosene (peak 13 in cuticular lipids from *M. rotundata* adult females; Fig. 1B) shows a prominent molecular ion at $M^+ = 472$ and major cleavage ions at m/z 229 and m/z 243 (Fig. 2A). Conversely, the dimethyl disulfide derivative of 7-positional isomer of heptacosene (peak 14, Fig. 1B) shows a clearly different fragmentation pattern: major cleavage ions at m/z 145 and m/z 327 (Fig. 2B). Calculations for the percent composition of mono-alkene positional isomers for each chain length (Table 2) were based on the relative abundance of the major mass spectral peak for the dimethyl disulfide derivative.

The occurrence and distribution for C_{23-33} mono-alkenes and their positional isomers varied greatly for *O. lignaria* and *M. rotundata* female adults (Table 2). For *O. lignaria*, there were 14 identified alkene compounds. In terms of percent composition for alkenes from *O. lignaria* females, the major compound was 9-heptacosene: constituting 48.5% of the total alkenes. Other major alkene constituents were 7-nonacosene (18.4%), 7-heptacosene (8.0%), 7-pentacosene (7.8%) and 9-pentacosene (7.1%). Comparatively, there were twice as many *M. rotundata* mono-alkenes as *O. lignaria* mono-alkenes. The major mono-alkene constituent of *M. rotundata* was 7-pentacosene (30.6%) with lesser amounts of 9-pentacosene (13.4%), 7-heptacosene (13.2%), 5-heptacosene (6.5%) and 13-heptacosene (6.4%).

For both species, the chain-length distributions for both alkanes and summed mono-alkenes were compared (Fig. 3). For *M. rotundata*, the abundance of alkanes decreased as the chain length increased ($C_{25:0} > C_{27:0} > C_{29:0}$, etc.) and this abundance pattern was also observed for the *M. rotundata* mono-alkenes. The alkanes of the *O. lignaria* females also decreased as the chain length increased, but for their mono-alkenes, the longer carbon chain $C_{27:1}$ and $C_{29:1}$ constituents were more abundant than the $C_{25:1}$ constituents (Fig. 3).

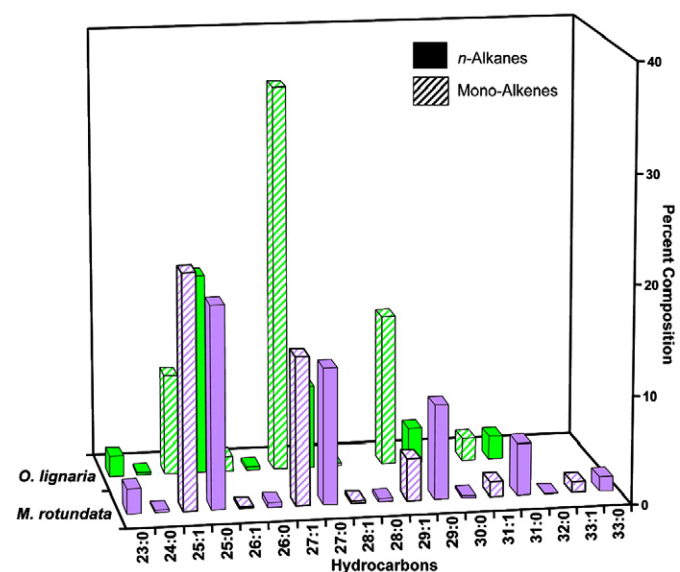


Fig. 3. Comparison of the *n*-alkane and summed mono-alkene chain-length distributions in the cuticular lipids from *O. lignaria* and *M. rotundata* adult females. Percentage compositions for the *n*-alkanes and summed mono-alkenes were calculated from the data shown in Table 1. The numbers to the left and the right of the colon for hydrocarbons are the number of carbons and the number of double bonds, respectively.

4. Discussion

Behavioral and chemical evidence show that *O. lignaria* and *M. rotundata* females mark their nests with olfactory cues that they use to recognize their nest and distinguish it from others in nesting aggregations (Guédot et al., 2005, 2006). Females of these same species have also been found to be attracted to certain materials from old nest cavities (Pitts-Singer, 2007). In our search to find the exact identity of nest recognition or attraction cues, and their origins, this analysis of the complete composition of female cuticular lipids of *O. lignaria* and *M. rotundata* examines possible sources of recognition or attraction cues. Furthermore, solitary bee cuticles have also been reported to contain the species' sex pheromone (Paulmier et al., 1999; reviewed in Ayasse et al., 2001), and this study could help to further our understanding of mating behavior in these two Megachilid species. We were able to identify completely and quantify the cuticular lipids from newly emerged individual *O. lignaria* and *M. rotundata* females to compare the mean composition differences between these two bee species.

The chromatographic cuticular lipid profiles for *O. lignaria* and *M. rotundata* (Fig. 1A and B, respectively), show species-specific differences. The cuticular lipids of *O. lignaria* consisted of unusually high quantities of alkenes, and 49.7% of the cuticular lipids were C_{27} and C_{29} mono-alkenes (Table 1), and for the 14 *O. lignaria* mono-alkenes, 9-heptacosene and 7-nonacosene comprised 67% of the total alkene distribution (Table 2). Conversely, the *M. rotundata* C_{27} *n*-alkane average quantity was nearly equal to the quantities of C_{27} mono-alkenes and, for the C_{29} components; the *n*-alkane amount was twice that of the mono-alkenes. The mixtures of mono-alkenes for *M. rotundata* females were more complex with 28 constituents identified and quantified and 57% of the total composition consisting of the three alkenes, 7-pentacosene, 9-pentacosene and 7-heptacosene (Table 2). The differences in the distribution of saturated hydrocarbons to unsaturated hydrocarbons for the two Megachilid species are shown in Fig. 3. The pattern for distribution of the homologous series of major alkanes (C_{25} , C_{27} , C_{29} , C_{31}) for both *O. lignaria* and *M. rotundata* was the same: a decrease with increased carbon chain length ($C_{25} > C_{27} > C_{29}$). The same pattern of decrease with increased chain length was shown for the mono-alkenes of *M. rotundata*. However, the mono-alkenes of *O. lignaria* did not show that pattern. The major carbon chain-length alkenes were C_{27} , C_{29} , C_{25} and C_{31} in decreasing order of abundance. The alkene compositions for *O. lignaria* and *M. rotundata* female cuticles appear to fall within the expected ranges and isomer distributions as reported for other insects. In addition to saturated *n*-alkanes, olefins (*n*-alkenes) are found in many insects, and frequently occur as mixtures of positional isomers (Blomquist et al., 1987; Lockey, 1988; de Renobales et al., 1991); and most olefin mixtures contain alkenes (mono-alkenes), whereas alkadienes and alkatrienes are less common (Lockey, 1988).

The cuticular lipids from both *O. lignaria* and *M. rotundata* females contained small quantities of wax esters and methyl-branched alkanes. The wax esters ranged in carbon chain length from C_{40} to C_{48} (Table 1, Fig. 1). These wax esters were unique in that the major fatty acid moiety for each carbon chain length was the unsaturated fatty acid, 9-octadecenoic acid (oleic acid). The occurrence of insect wax esters with unsaturated fatty acid moieties is not very common, but alkanyl alkenoates have been reported as constituents in the cuticular lipids of some insect species: flour beetle larva, *Tenebrio molitor* L. (Coleoptera: Tenebrionidae) (Bursell and Clements, 1967); three species of dragonflies (Jacob and Hanssen, 1979); and adult honey bee (Blomquist et al., 1980). Small amounts of C_{25} – C_{29} odd-carbon number mono-methyl-branched alkanes were identified in the cuticular lipids of *M. rotundata* and small quantities of C_{23} – C_{33} in *O. lignaria* (Table 1). A similar occurrence for dimethyl-branched alkanes was reported for *M. rotundata* females by Paulmier et al. (1999). The occurrence of methyl-branched lipids in insects has been thoroughly

reviewed, including mono-methyl- and multiple-methyl-branched alkanes (Nelson, 1993).

Cuticular lipid compositions have previously been reported for several hymenopteran bee species. For numerous social bee species, substantial amounts of alkenes are present in the cuticular hydrocarbons. In the adult honey bee, *Apis mellifera* L. (Apidae), dramatic differences were observed in the hydrocarbons of bees of different age. From day 7 to day 26, long carbon chain alkanes increased in amount with a corresponding decrease in the quantities of C_{23} – C_{33} mono-alkenes (Blomquist et al., 1980). Alkenes have been reported as major components of the cuticular lipids of bumblebee species. In *Bombus appositus* Cresson (Apidae), C_{21} – C_{29} 7-mono-alkenes comprised 48% of the cuticular hydrocarbons, and 35% of *B. occidentalis* Greene cuticular hydrocarbons were C_{23} – C_{31} 9-mono-alkenes (Hadley et al., 1981). For *B. protorum* L., *B. pascuorum* Scopoli, and *B. lapidarius* L., C_{23} and C_{25} mono-alkenes were found in the same proportions as alkanes, but for *B. occidentalis*, the C_{29} alkene, 9-nonacosene, accounted for 22% of the cuticular lipids (Oldham et al., 1994).

Although only a few species of solitary bees have been analyzed for their cuticular lipid composition, their lipids are similar in composition to those of social bees and to those of the two bee species in our study. The ground-nesting *Nomia bakeri* Cockerell (Halictidae) was found to be unique in that the female hydrocarbons were composed almost entirely of *n*-alkanes (Hadley et al., 1981). Analyses of the cuticular hydrocarbons of the burrowing bee, *Amegilla dawsoni* Rayment (Apidae: Apinae: Anthophorini), revealed the presence of *n*-alkanes and mono-alkenes along with fatty acids that differed in their relative amounts depending on the bee's sex and mating status (Simmons et al., 2003). Revealing more chemical complexity are the cuticles of the ground-nesting bee *Colletes cunicularius* (L.) (Colletidae) that consist of aldehydes and esters, in addition to the expected alkanes and alkenes all of which also differed according to bee sex and mating status (Mant et al., 2005).

The overall composition for the cuticular hydrocarbons of *M. rotundata* in this study is comparable to that reported for young *M. rotundata* females (Paulmier et al., 1999), in which the cuticular components were identified as *n*-alkanes, mono-alkenes, and fatty acids. However, in our study, the ratios of alkanes to alkenes were consistently higher for the C_{25} – C_{29} chain lengths, and fatty acids were not cuticular lipid constituents. In the Paulmier et al. (1999) study, the presence of wax esters was not reported, and the presence of substantial amounts of fatty acids could possibly have arisen from internal lipid contamination as a result of a long extraction time (24 h) in more polar organic solvents (i.e., CH_2Cl_2 or methanol). We have also correlated the occurrences of fatty acids on the cuticular surfaces of *M. rotundata* and *O. lignaria* females to contamination (Buckner and Pitts-Singer, unpublished).

The use of hydrocarbons for chemical communication, as a secondary function, has been well documented (Nelson, 1978; Blomquist and Jackson, 1979; Hadley, 1981; Howard and Blomquist, 1982; Blomquist and Dillwith, 1985; Blomquist et al., 1987; Lockey, 1988; de Renobales et al., 1991; Buckner, 1993; Howard, 1993; Nelson and Blomquist, 1995). The possible semiochemical roles of cuticular hydrocarbons as sex pheromones, species-, colony- and mate-recognition cues, as well as anti-aphrodisiacs, have been reviewed for both solitary and social insects (Howard, 1993; Singer, 1998). Cuticular hydrocarbons (mainly methyl-branched alkanes) as nest recognition chemicals have been reported for several species of social wasps (Espelie and Hermann, 1990; Brown et al., 1991; Espelie et al., 1994; Singer et al., 1998). Cuticular alkenes have been suggested by Paulmier et al. (1999) as sex pheromones in *M. rotundata*. They reported that variations in the mono-alkenes allowed for distinction between young virgin females, which possessed more 7-pentacosene and 9-pentacosene, and older females, which had more 5-monoenes; male mate-seeking and copulatory activity was stimulated only by young female monoenes.

The present findings on the characterization of chemicals from the cuticles of female *M. rotundata* and *O. lignaria* bees can now be compared in future studies with chemicals characterized from Dufour's glands and old nest materials. Identified compounds can be used for conducting future laboratory and field testing of selected components and component blends for determining whether, and which, chemicals mediate bee nesting behavior.

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